

FORM PTO-1390 (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NO. 03037.00003
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known) 37 C.F.R. 1.51 09/914213
INTERNATIONAL APPLICATION NO. PCT/US00/04642	INTERNATIONAL FILING DATE February 24, 2000	PRIORITY DATE CLAIMED February 24, 1999
TITLE OF INVENTION ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION		
APPLICANT(S) FOR DO/EO/US Lynn M. ADAMS, Pamela B. DAVIS and Jianjie MA		
<p>Applicant herewith submits to the United State Designated/Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (PCT Article 31). <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). Specification (20 pp.); Claims 1-34 (4 pp.), 4 sheets of Drawings, Sequence Listing (1 p.), Abstract (1 p.) <input checked="" type="checkbox"/> has been communicated by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371 (c)(2)). <ol style="list-style-type: none"> <input type="checkbox"/> is attached hereto. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). <input type="checkbox"/> have been communicated by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). <input type="checkbox"/> An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11-20 below concern other document(s) or information included:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98. <input type="checkbox"/> An Assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included. <input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification. <input type="checkbox"/> A change of power of attorney and/or address letter. <input checked="" type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).. <input checked="" type="checkbox"/> Other items or information: PCT/RO/101 (6 p.); PCT Chapter II Demand (5 pp.) Copy of WO 00/50591 published August 31, 2000 w/PCT/ISA/210; Copy of Statement Accompanying Sequence Listing dated May 19, 2000 w/Sequence Listing (3 pp. in dup.); PCT/IPEA/416(1 p.); PCT/IPEA/409 (7 pp.); Response to Invitation to Correct Defects dated June 26, 2000(9 pp.); PCT/RO/105(1 p.); PCT/RO/106 (4 pp.); PCT/DO/EO/901(a)(2 pp.); PCT/ISA/225 (3 pp.); PCT/IPEA/408 (7 pp.); PCT/IPEA/402(1 p.); PCT/IB/332 (1 p.); PCT/IB/308 (1 p.); PCT/RO/132 (1 p.); PCT/RO/138 (1 p.); PCT/IB/304 (1 p.); PCT/ISA/202 (1 p.); PCT/RO/102 (1 p.) 		

U.S. APPLICATION NO. 09/914213 <small>37 CFR 1.51</small>		INTERNATIONAL APPLICATION NO. PCT/US00/04642		ATTORNEY'S DOCKET NO. 03037.00003	
17. <input checked="" type="checkbox"/> The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1,000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$ 860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.455(a)(2)) paid to USPTO \$ 710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$ 690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$ 100.00				CALCULATIONS	PTO USE ONLY
				ENTER APPROPRIATE BASIC FEE AMOUNT = Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)). \$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	34 -20 =	14	X \$18.00	\$252.00	
Independent Claims	3 - 3 =	0	X \$ 80.00	\$	
Multiple dependent claims (if applicable)			X \$270.00	\$270.00	
TOTAL OF ABOVE CALCULATIONS =				\$1,382.00	
<input checked="" type="checkbox"/> Applicant claims small entity status See 37 CFR 1.27. The fees indicated below above are reduced by 1/2.				\$691.00	
SUBTOTAL =				\$	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	
TOTAL NATIONAL FEE =				\$691.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property.				\$	
TOTAL FEES ENCLOSED =				\$691.00	
				Amount to be:	
				refunded	\$
				charged	\$
a. <input type="checkbox"/> A check in the amount of \$_____ to cover the above fees is enclosed. b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. 19-0733 in the amount of \$691.00 to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0733. A duplicate copy of this sheet is enclosed. d. <input type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Banner & Witcoff, Ltd. Eleventh Floor 1001 G Street, N.W. Washington, D.C. 20001-4597 Telephone (202) 508-9100 Date: <u>August 23, 2001</u>					
				SIGNATURE <u><i>Michele A. Kagan</i></u> 47,600 for Sarah Kagan Sarah A. Kagan NAME 32,141 REGISTRATION NUMBER	

09/914213
JC05 Rec'd PCT/PTO 23 AUG 2001

Attorney Docket No. 03037.00003
International Application No. PCT/US00/04642

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

BOX PCT

Lynn M. ADAMS, et al.

**National Phase Application
PCT/US00/04642**

Filed: February 24, 2000

Serial No.: Unassigned

Group Art Unit: Unassigned

Filed: CONCURRENTLY HEREWITH

Examiner: Unassigned

For: ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

PRELIMINARY AMENDMENT

Assistant Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Preliminarily to the examination of the above-identified application, kindly amend the application as follows:

In the Specification:

Page 1, after the title, insert the following paragraph:

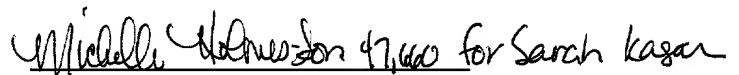
--This is a U.S. National Phase Application Under 35 USC 371 and applicant herewith claims the benefit of priority of PCT/US00/04642 filed February 24, 2000, which was published under PCT Article 21(2) in English and Application No. 60/121,495 filed in the United States on February 24, 1999.--

REMARKS

The amendment to the specification is made in accordance with 35 U.S.C. 119 and 37

C.F.R. 1.55 and 1.78. Entry is requested.

Respectfully submitted,


Sarah A. Kagan
Reg. No. 32,121

August 23, 2001
BANNER & WITCOFF, LTD.
Eleventh Floor
1001 G Street, N.W.
Washington, D.C. 20001-4597
(202) 508-9100

09/914213

JCO5 Rec'd PCT/PTO 23 AUG 2001

Attorney Docket No. 03037.00003
International Application No. PCT/US00/00938

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

BOX PCT

Lynn M. ADAMS, et al.

National Phase Application
PCT/US00/04642
Filed: February 24, 2000

Serial No.: Unassigned

Group Art Unit: Unassigned

Filed: CONCURRENTLY HEREWITH

Examiner: Unassigned

For: ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

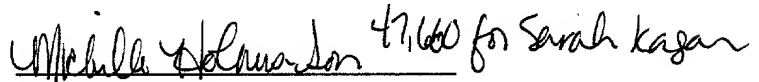
LETTER PURSUANT TO 37 CFR 1.821(f)

Assistant Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

In the matter of the above-identified application, which is filed concurrently herewith, Applicants submit a computer diskette containing the sequences of the instant application. It is hereby certified that the paper and computer copies of these sequences are identical in content.

Respectfully submitted,


Sarah A. Kagan
Reg. No. 32,141

August 23, 2001
BANNER & WITCOFF, LTD.
Eleventh Floor
1001 G Street, N.W.
Washington, D.C. 20001-4597
(202) 508-9100

SEQUENCE LISTING

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PCT09

RAW SEQUENCE LISTING
 PATENT APPLICATION: US/09/914,213

DATE: 09/13/2001
 TIME: 17:12:22

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 7 Function
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 C--> 12 <141> CURRENT FILING DATE: 2001-08-23
 14 <150> PRIOR APPLICATION NUMBER: 60/121,495
 15 <151> PRIOR FILING DATE: 1999-02-24
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RAW SEQUENCE LISTING

DATE: 09/13/2001

PATENT APPLICATION: US/09/914,213

TIME: 17:12:22

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RAW SEQUENCE LISTING

DATE: 09/13/2001

PATENT APPLICATION: US/09/914,213

TIME: 17:12:22

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VERIFICATION SUMMARY

DATE: 09/13/2001

PATENT APPLICATION: US/09/914,213

TIME: 17:12:23

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L:11 M:270 C: Current Application Number differs, Replaced Current Application Number

L:12 M:271 C: Current Filing Date differs, Replaced Current Filing Date

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Our File No.: 3037.86704

IN THE EUROPEAN PATENT OFFICE

International Application No. PCT/US00/04642	International Filing Date 24 February 2000	Priority Date Claimed 24 February 1999
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Title of Invention
ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

Applicant(s)
CASE WESTERN RESERVE UNIVERSITY

VIA FEDERAL EXPRESS

Zorka Bota, Authorized Officer
EUROPEAN PATENT OFFICE
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
NETHERLANDS

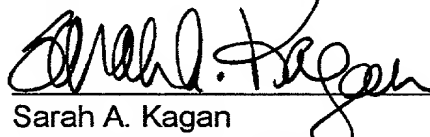
Attn.: ISA/EP

STATEMENT ACCOMPANYING SEQUENCE LISTING

Dear Sir:

The undersigned hereby states that the Sequence Listing submitted concurrently herewith does not include matter which goes beyond the content of the application as filed and that the information recorded on the diskette submitted concurrently herewith is identical to the written Sequence Listing.

Respectfully submitted,



Sarah A. Kagan
Agent for the Applicants
Reg. No. 32,141

Date: 19 May 2000

BANNER & WITCOFF, LTD.
1001 G STREET, N.W.
ELEVENTH FLOOR
WASHINGTON, D.C. 20001-4597
UNITED STATES OF AMERICA
(202) 508-9100

SEQUENCE LISTING

<110> Case Western Reserve University, School of Medicine

<120> Enhancers of CFTR Chloride Channel
Function

<130> 03037.86704

<140> pct/us 00/04624

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<170> FastSEQ for Windows Version 3.0

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ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

This invention was made with government support under RO1 HL/DK 49003, P30 DK27651 and RO1 DK51770 awarded by the National Institute of Health. The government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

This invention is related to the field of cystic fibrosis. More particularly, it is related to the area of therapeutic treatments and drug discovery for treating cystic fibrosis.

BACKGROUND OF THE INVENTION

Defects in CFTR, a chloride channel located in the apical membrane of epithelial cells, are associated with the common genetic disease, cystic fibrosis (Quinton, 1986, Welsh and Smith, 1993, Zielenski and Tsui, 1995). CFTR is a 1480 amino acid protein that is a member of the ATP binding cassette (ABC) transporter family (Riordan et al., 1989, Higgins, 1992). Each half of CFTR contains a transmembrane domain and a nucleotide binding fold (NBF), and the two halves are connected by a regulatory, or R domain. The R domain is unique to CFTR and contains several consensus PKA phosphorylation sites (Cheng et al., 1991, Picciotto et al., 1992).

Opening of the CFTR channel is controlled by PKA phosphorylation of

serine residues in the R domain (Tabcharani et al., 1991, Bear et al., 1992) and ATP binding and hydrolysis at the NBFs (Anderson et al., 1991, Gunderson and Kopito, 1995). Phosphorylation adds negative charges to the R domain, and introduces global conformational changes reflected by the reduction in the α -helical content of the R domain protein (Dulhanty and Riordan, 1994). Thus, electrostatic and/or allosteric changes mediated by phosphorylation are likely to be responsible for interactions between the R domain and other CFTR domains that regulate channel function (Rich et al., 1993, Gadsby and Nairn, 1994).

Rich et al., 1991 showed that deletion of amino acids 708-835 from the R domain (Δ R-CFTR), which removes most of the PKA consensus sites, renders the CFTR channel PKA independent, but the open probability of Δ R-CFTR is one-third that of the wild type channel and does not increase upon PKA phosphorylation (Ma et al., 1997, Winter and Welsh, 1997). Thus, it is possible that deletion of the R domain removes both inhibitory and stimulatory effects conferred by the R domain on CFTR chloride channel function. This conclusion is supported by studies that show that addition of exogenous unphosphorylated R domain protein (amino acids 588-858) to wt-CFTR blocks the chloride channel (Ma et al., 1996), suggesting that the unphosphorylated R domain is inhibitory. Conversely, exogenous phosphorylated R domain protein (amino acids 588-855 or 645-834) stimulated the Δ R-CFTR channel, suggesting that the phosphorylated R domain is stimulatory (Ma et al., 1997, Winter and Welsh, 1997). Therefore, it appears that the manifest activity (stimulatory or inhibitory) depends on the phosphorylation state of the R domain.

About 25% of the known 700 mutations in CFTR produce a mutant CFTR

protein which is properly transported to the apical membrane of epithelial cells but have only low level, residual channel activity. There is a need in the art for agents which can boost the level of channel activity in those mutants having low level activity.

5 **SUMMARY OF THE INVENTION**

It is an object of the present invention to provide an isolated polypeptide useful for enhancing the open probability of CFTR chloride channels.

It is another object of the present invention to provide a method of activating a CFTR protein to enhance its open probability.

10 These and other objects of the invention are achieved by providing one or more of the embodiments described below. In one embodiment of the invention an isolated polypeptide is provided. The polypeptide comprises a portion of CFTR (cystic fibrosis transmembrane conductance regulator) protein of between 10 and 100 amino acids, said portion comprising 18 amino acids as shown in SEQ ID NO:

15 1.

In another embodiment of the invention a method is provided for activating a CFTR protein. A polypeptide is applied to a CFTR protein which forms a cAMP regulated chloride channel. The polypeptide consists of a portion of CFTR protein which comprises 18 amino acids as shown in SEQ ID NO: 1, whereby the open
20 probability of the channel formed by the CFTR increases by at least 25%.

According to another aspect of the invention a method is provided for activating a CFTR protein. A polypeptide is applied to a CFTR protein which forms a cAMP regulated chloride channel. The polypeptide consists of a portion of CFTR protein which comprises 22 amino acids as shown in SEQ ID NO: 2, whereby the

open probability of the channel formed by the CFTR increases by at least 25%.

The present invention thus provides the art with reagents and tools for enhancing function of channels which are defective in cystic fibrosis patients.

DETAILED DESCRIPTION OF THE DRAWINGS

5 Figure 1. Deletion of Negatively Charged Regions from the R Domain Results in Expression of Mature Glycosylated, Phosphorylatable CFTR Proteins

(Figure 1A) Sequences of NEG1 and NEG2 within the R domain. Residues where mutations have been identified in the CFTR cDNA are underlined (E822K, E826K, D836Y).

10 (Figure 1B) NEG2 is predicted to form an amphipathic α -helix as determined by secondary structure determination (Geourjon and Deleage, 1995, Rost and Sander, 1993, Rost and Sander, 1994) and illustrated in this space filling model. Negatively charged residues are colored pink, and the positively charged lysine is colored green.

15 (Figure 1C) In vitro phosphorylation of wt-(lane 1), Δ NEG1- (lane 2) and Δ NEG2-CFTR (lane 3) by PKA in the presence of γ - 32 P-ATP. Both the core (band B) and fully glycosylated (band C) forms of all three CFTR molecules are phosphorylated.

Figure 2. Δ NEG2-CFTR Forms a Chloride Channel that is Unregulated by PKA

20 (Figure 2A) Single channel currents of wt, Δ NEG1- and Δ NEG2-CFTR incorporated into the lipid bilayer. While activities of wt-and Δ NEG1-CFTR absolutely require the presence of PKA in the *cis*-intracellular solution, the Δ NEG2-CFTR channel opens without PKA phosphorylation.

(Figure 2B) Diary plot of Δ NEG2-CFTR channel open probability versus time shows

that addition of 200 units/ml of PKA, a maximally stimulating concentration, does not affect channel activity. The dashed line indicates the average open probability for each segment of the experiment. Channels were recorded at -100 mV.

Figure 3. The Synthetic NEG2 Peptide both Stimulates and Inhibits CFTR

(Figure 3A) Diary plot (open probability versus time) of a wt-CFTR channel illustrating the effect of the NEG2 peptide on the open probability of the channel in the planar lipid bilayer. The concentration of synthetic NEG2 in the *cis*-intracellular solution is indicated above the plot.

(Figure 3B) Single channel currents from the wt-CFTR channel were acquired at -80 mV at the points indicated in A. The *cis*-intracellular solution contained 2 mM ATP and 50 units PKA/ml.

(Figure 3C) Single channel trace from Δ NEG2-CFTR incorporated into the lipid bilayer membrane. Traces were acquired at -80 mV. The *cis*-solution contained 2 mM ATP and no PKA. The top two traces were acquired before synthetic NEG2 peptide addition, with the second trace being an expansion of the first. In the bottom two traces, 0.44 μ M of the NEG2 peptide has been added and stimulation is observed. The closed time visibly decreases after peptide addition.

Figure 4. NEG2 Enhances CFTR Channel Activity by Increasing the Opening Rate of the Channel

Histograms of open and closed events of the wt-CFTR channel at -80 mV were generated without peptide (control, left panel) and with 4.4 μ M NEG2 peptide in the *cis*-solution (right panel).

(Figure 4A) The open time histograms contain a single exponential component with a time constant of 124 ms (control) and 105 ms (peptide-stimulated).

(Figure 4B) The closed time histograms contain a fast component and multiple slow components.

(Figure 4C) The closed-burst duration histograms were constructed using a delimiter of 40 ms (represented by the arrow in B). The solid lines in C represent the fit according to the double exponential equation $y = P_2 \cdot \exp[-t/\tau_2] + P_3 \cdot \exp[-t/\tau_3]$ where $\alpha_2 = 1/\tau_2$, $\alpha_3 = 1/\tau_3$, P_2 = probability of the intermediate closed component, and P_3 = probability of the long closed component. The best fit parameters are $P_2 = 0.811$, $\tau_2 = 459$ ms, $P_3 = 0.189$, $\tau_3 = 2494$ ms (control); $P_2 = 0.957$, $\tau_2 = 105$ ms, $P_3 = 0.043$, $\tau_3 = 1652$ ms (peptide-stimulated).

DETAILED DESCRIPTION OF THE INVENTION

It is a discovery of the present inventors that negatively charged amino acids at the carboxyl terminal of the R domain (817-838, NEG2) is involved in both the stimulatory and inhibitory functions of the R domain on the chloride channel. Moreover, a polypeptide which contains this portion of the CFTR amino acid sequence can be used to enhance the open probability of both wild-type and minimally active mutant CFTR protein.

The isolated polypeptide according to the invention consists of a portion of CFTR (cystic fibrosis transmembrane conductance regulator) protein. The portion preferably contains at least 18 amino acids as shown in SEQ ID NO: 1. However, fewer amino acid residues of the sequence may be used if they retain the channel enhancing function described herein for the 18 and 22 residue polypeptides. See also SEQ ID NO: 2. Thus the polypeptide may be from about 10 or 15 amino acid residues up to about 30 or even 100 amino acid residues. An isolated polypeptide may be synthetic or made in a

recombinant organism. It may be a proteolytic cleavage product of a larger primary expression product, including full-length, wild-type CFTR. Preferably the polypeptide will be free of full-length CFTR. The polypeptide will preferably be free of other proteins and polypeptides as well. However, it may be desirable that the polypeptide be fused to another polypeptide to provide additional functional properties. For example, fusion to another protein such as keyhole limpet hemocyanin would be used to increase immunogenicity. Another desirable fusion partner is a membrane-penetrating peptide. Such peptides include VP-22 (SEQ ID NO: 3), as well as the peptides shown in SEQ ID NO: 4 and SEQ ID NO: 5. Such peptides can be used to facilitate the uptake by target cells of the polypeptide.

The polypeptides of the present invention can be used to enhance the function of wild-type or minimally active mutant CFTR proteins. The polypeptide functions to decrease the closed time of the channels formed by CFTR. A polypeptide can be applied to the CFTR protein in any context. It can be applied *in vitro* or *in vivo*. If *in vitro* it can be to CFTR in cultured cells or to planar bilayer membranes containing CFTR. If *in vivo*, the polypeptide can be applied directly to airway epithelial cells. Such application can be by any means known in the art, including but not limited to using a gargle or a nebulizer to deliver aerosolized polypeptide to the target cells. In addition, the peptide can be delivered in an indirect mode, by delivering a gene construct to the airway epithelial cells, which when taken up by the cells causes them to express the polypeptide. The delivery of the polypeptide to the CFTR preferably increases the open probability of the channel formed by the CFTR by at least 25%. More preferably it increases the open probability by at least 50%, at least 75%, at least 100%, at least 125%, at least 150%, or at least 200%.

A CFTR construct comprises a nucleic acid sequence encoding the amino acid sequence shown in SEQ ID NO: 1. A suitable promoter for expression in lung epithelia is also desirable. Many such promoters are known in the art, and any can be used as appropriate for a particular application.

5 It is believed that the administration of the polypeptide of the present invention will be the most useful in treatment of a class of mutants which produce CFTR proteins which are properly delivered to the plasma membrane but which are only residually or minimally active. Known mutants of CFTR are listed at <http://www.genet.sickkids.on.ca/cftr-cgi-bin/fulltable>. One can determine that a
10 particular CFTR mutant is fully processed and reaches the plasma membrane in a Western blot assay using antibody against CFTR. Fully processed mutants achieve mature glycosylation status and appear on the gel as "band C and band B" whereas mutants that are retained in the endoplasmic reticulum are not fully glycosylated and show only "band B". See Example 2, below and Figure 1C.

15 The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLES

20 *Example 1: Deletion of a negatively charged region (a.a. 817-838) from the R domain of CFTR alters PKA-dependent regulation of the CFTR channel.*

CFTR contains a large intracellular regulatory (R) domain where multiple PKA phosphorylation sites are located. There are two regions within the R domain that

contain a high proportion of negatively charged amino acids, a.a. 725-733 (NEG1) and a.a. 817-838 (NEG2). It is possible that these two regions could have allosteric or electrostatic interactions with other regions of CFTR and thus affect its chloride channel function. To test the role of NEG1 and NEG2, two deletion mutants, NEG1-CFTR and NEG2-CFTR, were created. The CFTR mutants were transiently expressed in HEK 293 cells, and their single channel functions were studied using the bilayer reconstitution system. Western blots indicate that both NEG1-CFTR and NEG2-CFTR process normally and traffic to the plasma membrane of HEK 293 cells. Both mutants form functional chloride channels in the bilayer membrane, with single channel conductances similar to the wt-CFTR channel. Like wt-CFTR, opening of NEG1-CFTR requires absolutely PKA phosphorylation and ATP binding/hydrolysis. In contrast to wt-CFTR, opening of NEG2-CFTR does not require PKA phosphorylation. Thus, deletion of NEG2, but not NEG1, alters PKA-dependent regulation of the CFTR chloride channel. Our data suggest that NEG2 could form a 'putative gating particle' of the CFTR channel possibly through electrostatic and/or allosteric interactions with other domains of CFTR.

Example 2: Δ NEG1- and Δ NEG2-CFTR are glycosylated.

The R domain of CFTR contains two negatively charged regions, amino acids 725-733 (NEG1) and amino acids 817-838 (NEG2), that reside in close proximity to two PKA phosphorylation sites, S737 and S813, used in vivo (Figure 1A) (Cheng, et al. 1991). NEG2 is predicted to form an amphipathic (α -helical structure with a negatively charged face (Figure 1B) (Geourjon and Deleage, 1995, Rost and Sander, 1993, Rost and Sander, 1994). Three mutations (E822K, E826K, D836Y), two of

which were clearly obtained from patients with CF (E822K and D836Y), have been identified within the NEG2 region that result in the removal of negative charges (www.genet.sickkids.on.ca). The E822K CFTR channel has a low open probability relative to wt-CFTR (wild type-CFTR), but the E826K CFTR channel has single channel properties similar to wt-CFTR (Vankeerberghen et al., 1998). The presence of these disease-causing mutations suggests the potential importance of the NEG2 region. To investigate the roles of NEG1 and NEG2 in CFTR function, these regions were deleted from CFTR using mutagenesis and subcloning. The Δ NEG1- and Δ NEG2-CFTR proteins were transiently expressed in human embryonic kidney 293 cells. Membrane vesicles containing the CFTR proteins were isolated and subjected to SDS-PAGE. Like wt-CFTR, both Δ NEG1- and Δ NEG2-CFTR are present both in the core glycosylated (band B) and the fully glycosylated form (band C) (Figure 1C).

Example 3: *The open probability of the Δ NEG2-CFTR chloride channel is much less than that of wild type but is independent PKA, although it contains all PKA phosphorylation sites.*

Single channel measurements indicate that the Δ NEG1-CFTR channel is similar to wt-CFTR in its PKA dependence. No chloride channels are observed in the absence of PKA (Figure 2A) and the open probability in the presence of PKA and ATP is similar to wt-CFTR. In contrast, the Δ NEG2-CFTR channel opens without PKA (Figure 2A). The constitutive activity of the Δ NEG2-CFTR channel is unlikely to be due to the endogenous phosphorylation of the Δ NEG2-CFTR protein, since protein phosphatase 2A, which decreases activity of the wt-CFTR opened by PKA and ATP (Ma et al., 1997), has no effect on the Δ NEG2-CFTR channel (n=4). Moreover, addition of PKA up to 200 units/ml, four times the concentration required to fully

activate wt-CFTR (Ma et al., 1997), does not increase the open probability of the channel (Figure 2B). Δ NEG2-CFTR has conductance properties similar to wild type (Tao et al., 1996). However, the open probability of the Δ NEG2-CFTR chloride channel is much less than that of wild type and cannot be increased by PKA ($P_o = 0.035$ (0.012 and $P_o = 0.026$ (0.013 without and with PKA respectively, $n=5$). While NEG2 may represent an inhibitory region, removal of these amino acids does not result in a fully activated channel. The failure of the Δ NEG2-CFTR channel to respond to PKA does not result from inability of the channel to be phosphorylated, for an in vitro assay using ($-^{32}\text{P}$ -ATP showed comparable phosphorylation of wt-CFTR and Δ NEG2-CFTR (Figure 1C). Thus, it appears that removal of NEG2 from CFTR completely eliminates the PKA dependence of the chloride channel, although the Δ NEG2-CFTR channel still contains all ten PKA sites and can be phosphorylated.

Example 4: *NEG2 polypeptide stimulates both wild-type and Δ NEG2 CFTR proteins at concentrations greater than 0.44 μM .*

To test whether the NEG2 region is responsible for both stimulatory and inhibitory interactions between the R domain and other domains, synthetic NEG2, a 22 amino acid peptide, was added to the cis-intracellular side of single CFTR channels captured in the planar lipid bilayer (Figure 3). The diary plot of open probability as a function of time shows the activity of a single wt-CFTR channel during the course of the experiment (Figure 3A). After peptide addition, there are periods of intense stimulation that last 4 to 8 minutes. These stimulatory periods are followed by either a return to the basal level of activity before peptide addition, or by an almost complete inhibition of the channel, where only a flickery 3 pS conductance is observed. During stimulation, the open probability more than doubles and more transitions are observed

between the open and closed states (Figure 3B). The stimulatory response was observed in 6 of 7 experiments at concentrations $\geq 0.44 \mu\text{M}$ (the remaining channel was inhibited upon peptide addition ($4.4 \mu\text{M}$) and no stimulation was seen). Profound inhibition was observed in three channels at concentrations $\geq 4.4 \mu\text{M}$. When the NEG2 peptide was added to the intracellular side of the $\Delta\text{NEG2-CFTR}$ channel, which lacks its own endogenous NEG2 sequence, a similar stimulatory response was observed (Figure 3C).

Example 5: *The NEG2 peptide decreases the closed time of the wild-type CFTR protein.*

In order to understand the mechanism responsible for the increase in open probability, the gating kinetics of wt-CFTR without peptide and during stimulation by synthetic NEG2 were analyzed. The open time distributions of the wt-CFTR did not change during peptide stimulation, as both control (without NEG2 peptide) and peptide-stimulated channels had an open lifetime of approximately 120 ms (Figure 4A). Thus, the increase in the open probability is not due to a change in the closing rate of the channel. However, the closed time distribution for the stimulated channel is clearly shifted to the left compared to the control channel (Figure 4B). There are three components to the closed state, a fast (τ_{c1}), an intermediate (τ_{c2}), and a long (τ_{c3}) closed component. The fast closed component is probably due to closings within a burst (Carson et al., 1995). Therefore, to identify better the closed times between bursts, a delimiter of $\tau_c = 40 \text{ ms}$ was set at the nadir between the fast and intermediate closed times (illustrated by the arrow in Figure 4B) to generate the closed-burst duration histograms. As shown in Figure 4C, following peptide stimulation, the intermediate closed time was reduced from 459 ms to 105 ms, whereas the long closed time

remained relatively unchanged. Thus, the interaction of NEG2 with CFTR increased the intermediate-opening rate of the channel. This increase in opening rate is similar to that observed when the phosphorylated R domain protein (amino acids 645-834) was added to CFTR- Δ R/S660A in excised, inside-out patches (Winter and Welsh, 1997).

5 Additionally, modification of C832, which resides within NEG2, by N-ethylmaleimide (NEM) results in irreversible stimulation of PKA-phosphorylated CFTR chloride channel activity (Cotten and Welsh, 1997), further emphasizing the importance of NEG2 in CFTR regulation.

10 These data, taken together, show that the NEG2 region confers both stimulatory and inhibitory functions of the R domain on the CFTR channel. When this region is deleted from CFTR, the resultant channel opens without PKA (loss of inhibitory function), but it never achieves open probability comparable to wild type even when phosphorylated with PKA (loss of stimulatory function). This same sequence, expressed as a peptide, results in stimulation of channel openings at lower
15 concentrations and profound inhibition of channel activity at higher concentrations, when added to the intracellular side of CFTR channels. It seems likely that this sequence interacts with CFTR at different sites on the nucleotide binding domains to either stimulate or inhibit channel openings. Phosphorylation of the R domain, in this model, changes its conformation and thus presents the NEG2 sequence better to the
20 stimulatory than the inhibitory site. A current model for channel opening is that phosphorylated channels open in response to ATP binding and hydrolysis at the first nucleotide binding fold (NBF1) (Gadsby and Naim, 1994, Ma and Davis, 1998). Since stimulation by NEG2 occurs by increasing channel openings, a likely site of stimulation is NBF1, though other models are possible.

METHODS USED IN EXAMPLES 1-5

Subcloning of CFTR gene

The wt, Δ NEG1-, and Δ NEG2-CFTR cDNAs were subcloned into an Epstein-Barr virus-based episomal eukaryotic expression vector, pCEP4 (Invitrogen, San Diego, CA), between the NheI and XhoI restriction sites. The Δ NEG1 and Δ NEG2 deletion mutants were created using the pALTER mutagenesis system and shuttled from pALTER into pCEP4 by substituting the corresponding fragment in pCEP4 wt-CFTR with the mutant fragment between the XhoI and BstZ171 restriction sites. The Δ NEG1-CFTR cDNA has 27 bases deleted (amino acids 725-733). The Δ NEG2-CFTR cDNA has 66 bases deleted (amino acids 817-838).

Expression of CFTR in HEK 293 cells

A human embryonic kidney cell line (293-EBNA HEK; Invitrogen) was used for transfection and expression of the CFTR proteins (Ma et al., 1997, Ma et al., 1996, Xie et al., 1995). The HEK-293 cell line contains a pCMV-EBNA vector, which constitutively expresses the Epstein-Barr virus nuclear antigen-1 (EBNA-1) gene product and increases the transfection efficiency of Epstein-Barr virus-based vectors. The cells were maintained in Dulbecco's Modified Eagle Medium with 10% FBS and 1% L-glutamine. Geneticin (G418, 250 (g/ml) was added to the cell culture medium to maintain selection of the cells containing the pCMV-EBNA vector. Lipofectamine reagent (Life Technologies, Inc) in Optimem media (serum-free) was used to transfect the HEK-293 cells with pCEP4(wt), pCEP4(Δ NEG1), or pCEP4(Δ NEG2). After 5 hours, serum was added to the media (10% final serum concentration). Twenty-four hours after transfection, the transfection media was replaced with fresh media. The

cells were harvested two days after transfection and microsomal membrane vesicles were prepared for single channel measurements in the lipid bilayer reconstitution system.

Vesicle preparation from transfected HEK 293 cells

5 HEK-293 cells transfected with pCEP4(CFTR) were harvested and homogenized using a combination of hypotonic lysis and Dounce homogenization in the presence of protease inhibitors (Ma et al., 1997, Ma et al., 1996, Xie et al., 1995). Microsomes were collected by centrifugation of postnuclear supernatant (4500 x g, 15 min) at 100,000 x g for 20 min and resuspended in a buffer containing 250 mM sucrose, 10 mM
10 HEPES, pH 7.2. The membrane vesicles were stored at -75°C until use.

In vitro phosphorylation of CFTR proteins

CFTR proteins isolated in membrane vesicles were bound to protein G agarose using a mouse monoclonal anti-human CFTR antibody (Genzyme). The protein G agarose was washed, and (γ - 32 P-ATP (10 Ci) and protein kinase A (~10 units/50l) was
15 added. Samples were incubated at 30°C for one hour during phosphorylation. Excess (γ - 32 P-ATP was removed, and SDS-PAGE sample buffer (200 mM Tris-Cl, pH 6.7, 9% SDS, 6% beta-mercaptoethanol, 15% glycerol, and 0.01% bromophenol blue) was added to denature CFTR and release it from the protein G agarose. The samples were subjected to electrophoresis on a 5% SDS-polyacrylamide gel, transferred to a
20 polyvinylidene difluoride membrane, and exposed to film.

Preparation of NEG2 peptides

The 22 amino acid peptide corresponding to NEG2 was custom made by Quality Controlled Biochemicals, Inc. The peptide was resuspended in water to a concentration of 1 mg/ml and pH was adjusted to a physiological range (7.2-7.4) using KOH and HCl.

5 The space filling model of the NEG2 peptide was generated, based on secondary structure predictions (Geourjon and Deleage, 1995, Rost and Sander, 1993, Rost and Sander, 1994), using the Insight II program from Molecular Simulations Incorporated.

Reconstitution of CFTR channels in lipid bilayer membranes

Lipid bilayer membranes were formed across an aperture of ~200 μ m diameter with a mixture of phosphatidylethanolamine:phosphatidylserine:cholesterol in a ratio of 5:5:1. The lipids were dissolved in decane at a concentration of 33 mg/ml. The recording solutions contained: cis (intracellular), 200 mM CsCl, 1 mM MgCl₂, 2 mM ATP, and 10 mM HEPES-Tris (pH 7.4); trans (extracellular), 50 mM CsCl, 10 mM HEPES-Tris (pH 7.4). Vesicles (1-4 μ m) containing either wild-type, Δ NEG1-, or
10 Δ NEG2-CFTR were added to the cis solution. The PKA catalytic subunit was present at a concentration of 50 units/ml in the cis solution unless noted otherwise. Single channel currents were recorded with an Axopatch 200A patch clamp unit (Axon Instruments). The currents were sampled at 1-2.5 ms/point. Single channel data analyses were performed with pClamp and TIPS softwares.

References

20 Anderson, M.P., Berger, H.A., Rich, D.P., Gregory, R.J., Smith, A.E., and Welsh, M.J. (1991). Nucleoside triphosphates are required to open the CFTR chloride channel. Cell

67, 775-784.

Bear, C.E., Li, C., Kartner, N., Bridges, R.J., Jensen, T.J., Ramjeesingh, M., and Riordan, J.R. (1992). Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell* 68, 809-818.

5 Carson, M.R., Travis, S.M., and Welsh, M.J. (1995). The two nucleotide-binding domains of cystic fibrosis transmembrane conductance regulator (CFTR) have distinct functions in controlling channel activity. *J. Biol. Chem.* 270, 1711-1717.

Cheng, S.H., Rich, D.P., Marshall, J., Gregory, R.J., Welsh, M.J., and Smith, A.E. (1991). Phosphorylation of the R domain by cAMP-dependent protein kinase regulates the CFTR chloride channel. *Cell* 66, 1027-1036.

10 Cotten, J.F. and Welsh, M.J. (1997). Covalent modification of the regulatory domain irreversibly stimulates cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* 272, 25617-25622.

Dulhanty, A.M. and Riordan, J.R. (1994). Phosphorylation by cAMP-dependent protein kinase causes a conformational change in the R domain of the cystic fibrosis transmembrane conductance regulator. *Biochemistry* 22, 4072-4079.

15 Gadsby, D.C. and Nairn, A.C. (1994). Regulation of CFTR channel gating. *Trends Biochem. Sci.* 19, 513-518.

Geourjon, C. and Deleage, G. (1995). SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *CABIOS* 11, 681-684.

20 Gunderson, K.L. and Kopito, R.R. (1995). Conformational states of CFTR associated with channel gating: the role of ATP binding and hydrolysis. *Cell* 82, 231-239.

Higgins, C.F. (1992). ABC transporters: from microorganisms to man. *Annu. Rev. Cell*

Biol. 8, 67-113.

Ma, J. and Davis, P.B. (1998). What we know and what we do not know about cystic fibrosis transmembrane conductance regulator. *Clinics in Chest Medicine* 19, 459-471.

Ma, J., Tasch, J.E., Tao, T., Zhao, J., Xie, J., Drumm, M.L., and Davis, P.B. (1996).

5 Phosphorylation-dependent block of cystic fibrosis transmembrane conductance regulator chloride channel by exogenous R domain protein. *J. Biol. Chem.* 271, 7351-7356.

Ma, J., Zhao, J., Drumm, M.L., Xie, J., and Davis, P.B. (1997). Function of the R domain in the cystic fibrosis transmembrane conductance regulator chloride channel.

10 *J. Biol. Chem.* 272, 28133-28141.

Picciotto, M.R., Cohn, J.A., Bertuzzi, G., Greengard, P., and Nairn, A.C. (1992).

Phosphorylation of the cystic fibrosis transmembrane conductance regulator. *J. Biol. Chem.* 267, 12742-12752.

15 Quinton, P.M. (1986). Missing Cl⁻ conductance in cystic fibrosis. *Am. J. Physiol.* 251, C649-C652.

Rich, D.P., Berger, H.A., Cheng, S.H., Travis, S.M., Saxena, M., Smith, A.E., and Welsh, M.J. (1993). Regulation of the cystic fibrosis transmembrane conductance regulator Cl⁻ channel by negative charge in the R domain. *J. Biol. Chem.* 268, 20259-20267.

20 Rich, D.P., Gregory, R.J., Anderson, M.P., Manavalan, P., Smith, A.E., and Welsh, M.J. (1991). Effect of deleting the R domain on CFTR-generated chloride channels. *Science* 253, 205-207.

Riordan, J., Rommens, J., Kerem, B.-S., Noa, A., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsic, N., Chou, J.-L., Drumm, M., Iannuzzi, M., Collins, F.,

and Tsui, L.-C. (1989). Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245, 1066-1073.

Rost, B. and Sander, C. (1993). Prediction of protein structure at better than 70% accuracy. *J. Mol. Biol.* 232, 584-599.

5 Rost, B. and Sander, C. (1994). Combining evolutionary information and neural networks to predict protein secondary structure. *Proteins* 19, 55-72.

Tabcharani, J.A., Chang, X.-B., Riordan, J.R. and Hanrahan, J.W. (1991). Phosphorylation-regulated Cl⁻ channel in CHO cells stably expressing the cystic fibrosis gene. *Nature* 352, 628-631.

10 Tao, T., Xie, J., Drumm, M.L., Zhao, J., Davis, P.B., and Ma, J. (1996). Slow conversions among subconductance states of cystic fibrosis transmembrane conductance regulator chloride channel. *Biophys. J.* 70, 743-753.

Vankeerberghen, A., Wei, L., Jaspers, M., Cassiman, J.-J., Nilius, B., and Cuppens, H. (1998). Characterization of 19 disease-associated missense mutations in the regulatory domain of the cystic fibrosis transmembrane conductance regulator. *Hum. Mol. Genet.* 7, 1761-1769.

15 Welsh, M.J. and Smith, A.E. (1993). Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 73, 1251-1254.

Winter, M.C. and Welsh, M.J. (1997). Stimulation of CFTR activity by its phosphorylated R domain. *Nature* 389, 294-296.

20 Xie, J., Drumm, M.L., Ma, J., and Davis, P.B. (1995). Intracellular loop between transmembrane segments IV and V of cystic fibrosis transmembrane conductance regulator is involved in regulation of chloride channel conductance state. *J. Biol. Chem.* 270, 28084-28091.

Zielenski, J. and Tsui, L.C. (1995). Cystic fibrosis: genotypic and phenotypic variations. *Annu. Rev. Genetics* 29, 777-807.

CLAIMS:

1. An isolated polypeptide comprising a portion of CFTR (cystic fibrosis transmembrane conductance regulator) protein of between 10 and 100 amino acids, said portion comprising 18 amino acids as shown in SEQ ID NO: 1.
- 5 2. The polypeptide of claim 1 which comprises 22 amino acids as shown in SEQ ID NO: 2.
3. The polypeptide of claim 1 wherein the polypeptide is fused to a membrane-penetrating peptide.
4. The polypeptide of claim 2 wherein the polypeptide is fused to a membrane-penetrating peptide.
- 10 5. The polypeptide of claim 3 wherein the membrane-penetrating peptide is selected from the group consisting of: VP-22 (SEQ ID NO: 3), (SEQ ID NO: 4), and (SEQ ID NO: 5).
6. The polypeptide of claim 4 wherein the membrane-penetrating peptide is selected from the group consisting of: VP-22 (SEQ ID NO: 3), (SEQ ID NO: 4), and (SEQ ID NO: 5).
- 15 7. The polypeptide of claim 1 which is free of phosphorylation.
8. A method of activating a CFTR protein comprising:
applying a polypeptide to a CFTR protein which forms a cAMP
20 regulated chloride channel, said polypeptide comprising a portion of CFTR protein of between about 10 and 100 amino acids, said portion comprising 18 amino acids as shown in SEQ ID NO: 1, whereby the open probability of the channel formed by the CFTR increases by at least 25%.

9. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 50%.
10. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 75%.
- 5 11. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 100%.
12. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 125%.
13. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 150%.
- 10 14. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 200%.
- 15 15. The method of claim 8 wherein the CFTR protein is a mutant which reaches a cell's plasma membrane but fails to undergo full activation.
16. The method of claim 15 wherein the CFTR protein is listed at <http://www.genet.sickkids.on.ca/cfr-cgi-bin/fulltable>.
17. The method of claim 8 wherein the step of applying is performed by administering an aerosolized polypeptide to a patient with a mutant CFTR protein.
- 20 18. The method of claim 8 wherein the CFTR protein is in a lipid bilayer and a change in conductance is measured upon applying the polypeptide.
19. The method of claim 8 wherein the step of applying the polypeptide is accomplished by administering a nucleic acid encoding the polypeptide to a patient who expresses the CFTR protein, whereby the polypeptide is

expressed.

20. The method of claim 19 wherein the nucleic acid is administered as an aerosol to the patient's airways.

21. A method of activating a CFTR protein comprising:

5 applying a polypeptide to a CFTR protein which forms a cAMP regulated chloride channel, said polypeptide comprising a portion of CFTR protein of between 10 and 100 amino acids, said portion comprising 22 amino acids as shown in SEQ ID NO: 1, whereby the open probability of the channel formed by the CFTR increases by at
10 least 25%.

22. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 50%.

23. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 75%.

15 24. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 100%.

25. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 125%.

20 26. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 150%.

27. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 200%.

28. The method of claim 21 wherein the CFTR protein is a mutant which reaches a cell's plasma membrane but fails to undergo full activation.

29. The method of claim 28 wherein the CFTR protein is listed at
<http://www.genet.sickkids.on.ca/cftr-cgi-bin/fulltable>.
30. The method of claim 21 wherein the step of applying is performed by
administering an aerosolized polypeptide to a patient with a mutant CFTR
protein.
31. The method of claim 21 wherein the CFTR protein is in a lipid bilayer and a
change in conductance is measured upon applying the polypeptide.
32. The method of claim 21 wherein the step of applying the polypeptide is
accomplished by administering a nucleic acid encoding the polypeptide to a
patient who expresses the CFTR protein, whereby the polypeptide is
expressed.
33. The method of claim 32 wherein the nucleic acid is administered as an aerosol
to the patient's airways.
34. The method of claim 8 or 21 wherein the polypeptide is free of
phosphorylation.

Fig. 1

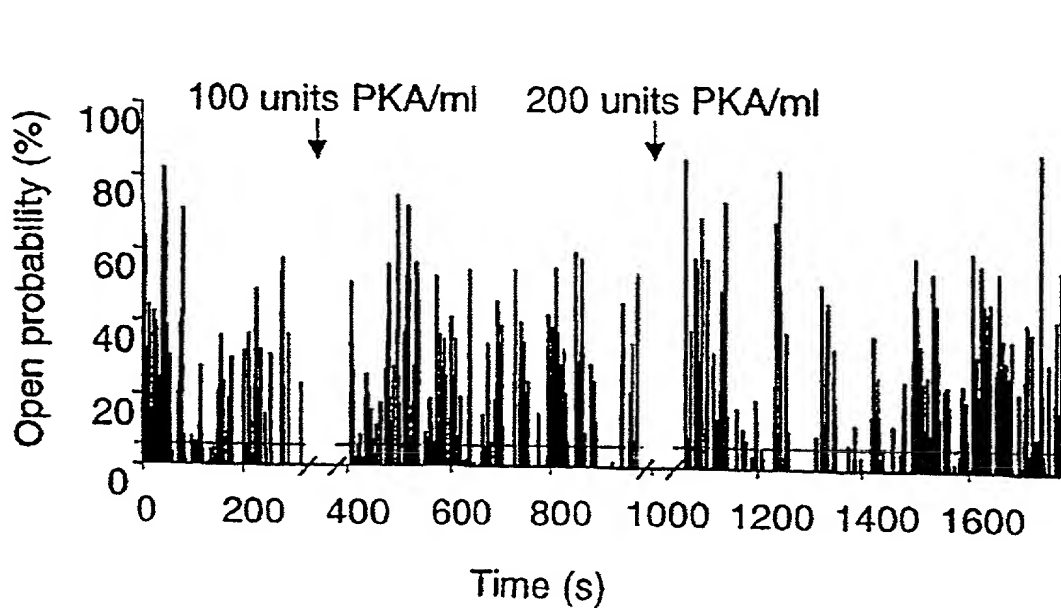
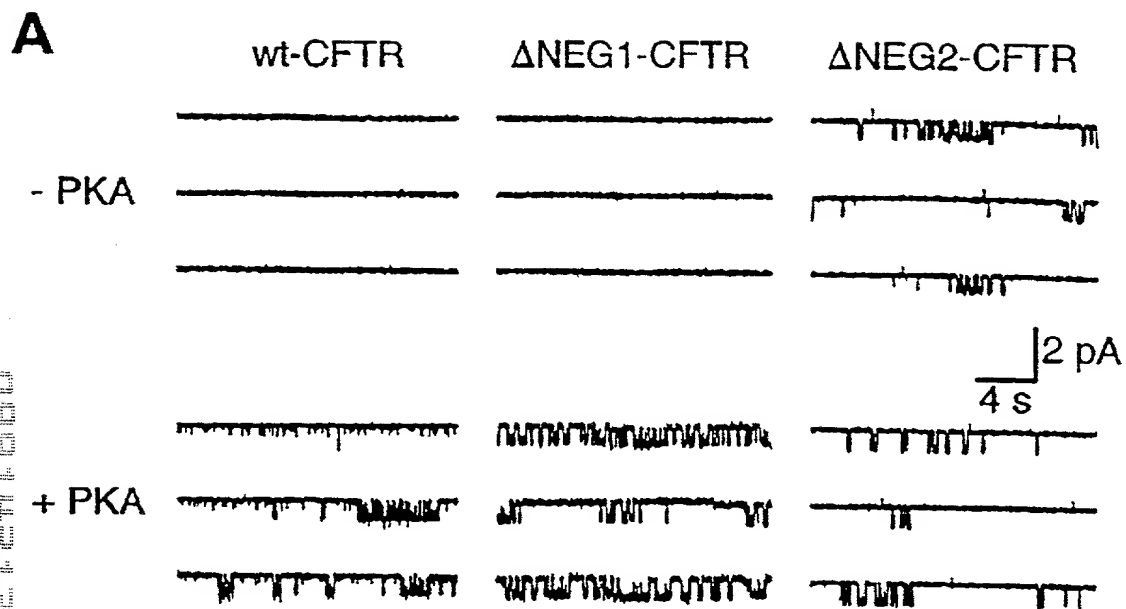


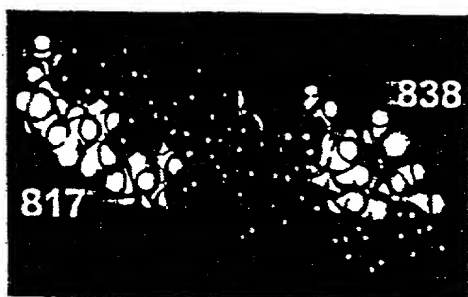
Fig. 2

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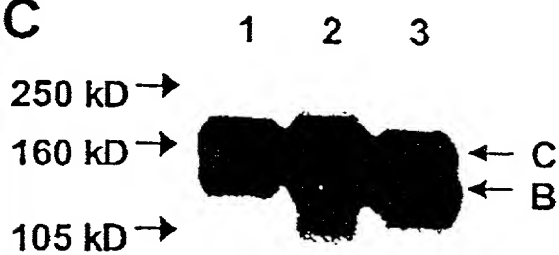
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B



C



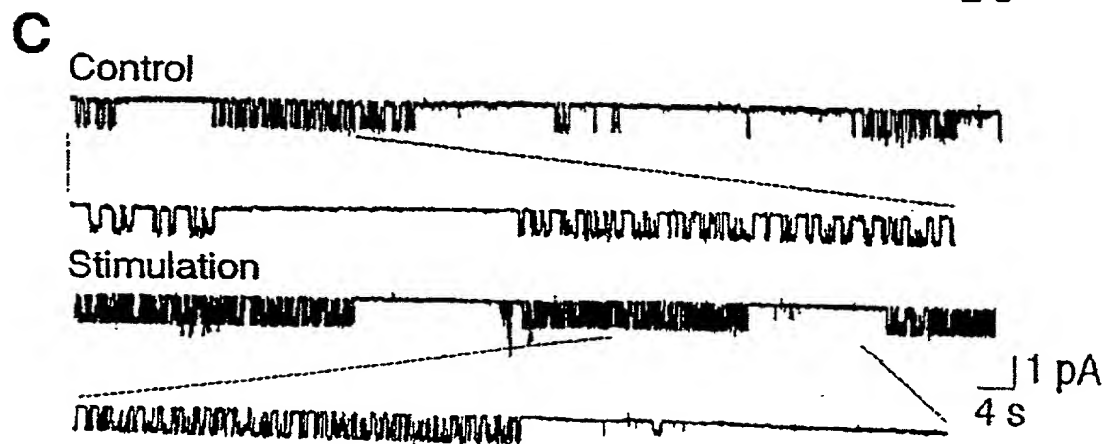
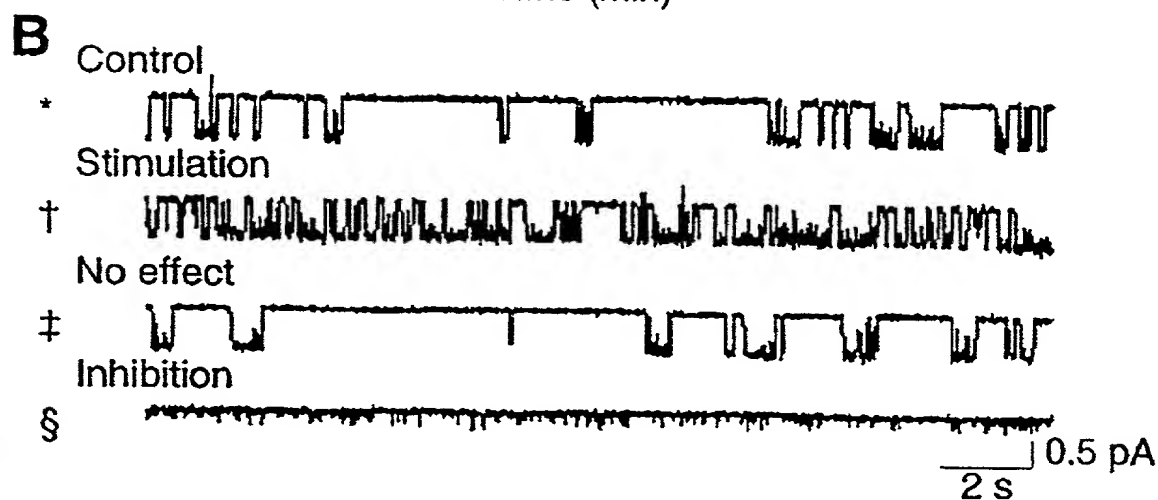
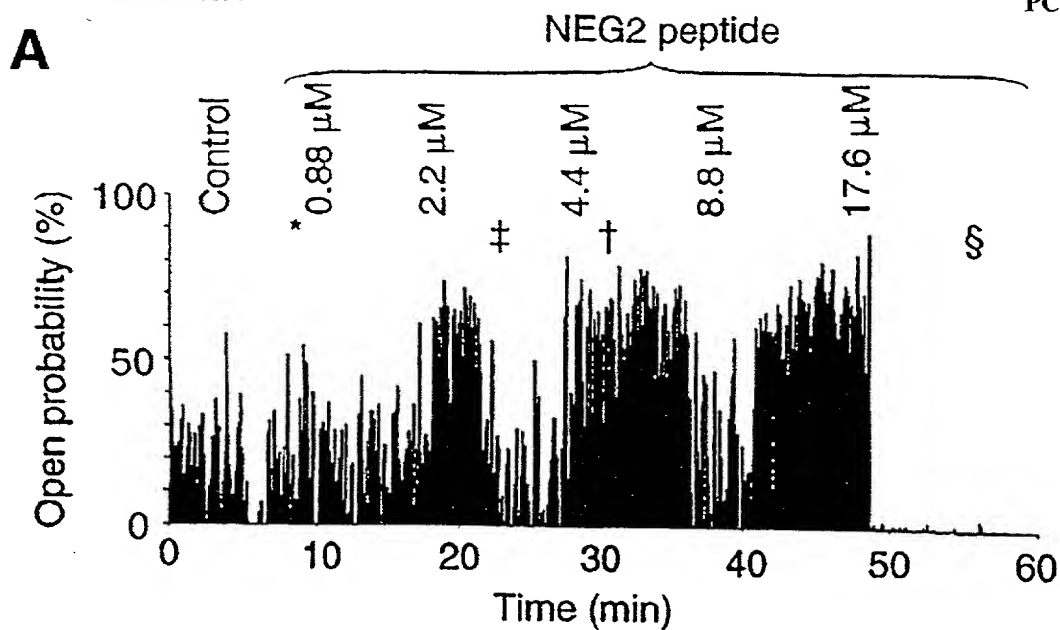
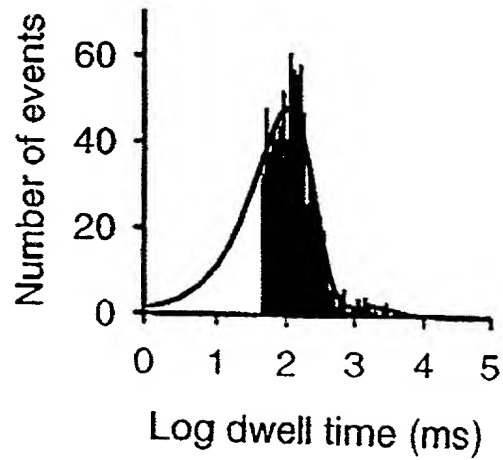
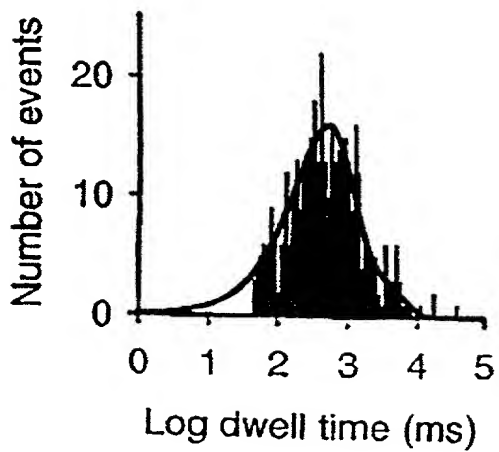
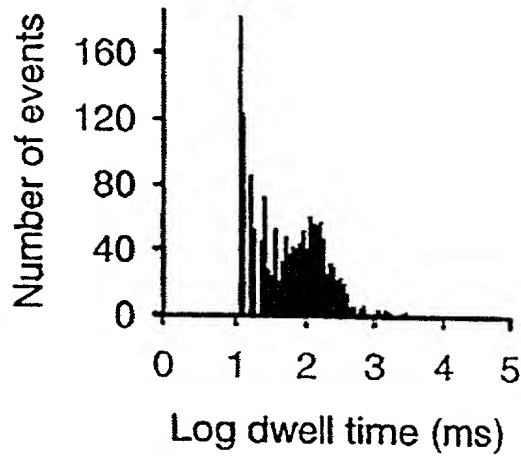
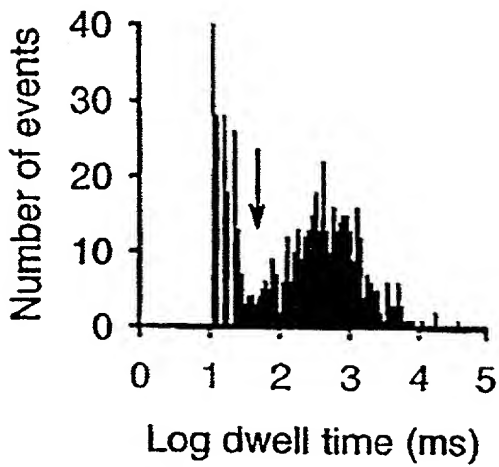
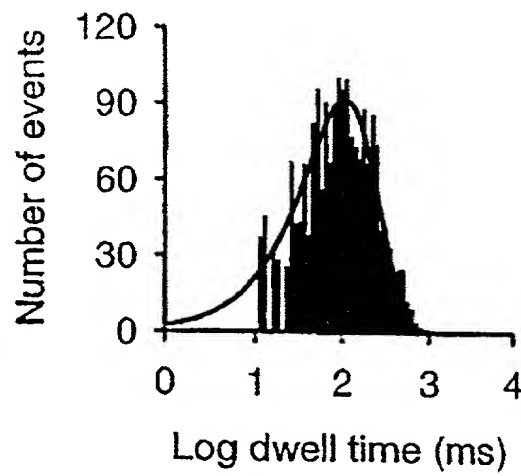
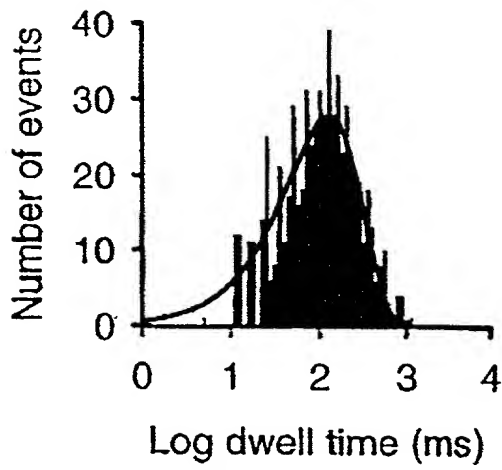


Fig. 4



Banner & Witcoff Ref No. 03037.00003

Client Ref. No.

JOINT DECLARATION FOR PATENT APPLICATION

As the below named inventors, we hereby declare that:

Our residence, post office address and citizenship are as stated below next to our names:

We believe we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION, the specification of which

- ☐ is attached hereto.
- ☒ was filed on August 23, 2001 as Application Serial Number 09/914,213 and was amended on August 23, 2001 (if applicable).
- ☒ was filed under the Patent Cooperation Treaty (PCT) and accorded International Application No. PCI/US00/04642, filed February 24, 2000, and amended on _____ (if any).

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We hereby acknowledge the duty to disclose information which is material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

Prior Foreign Application(s)

We hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Country	Application No.	Date of Filing (day month year)	Date of Issue (day month year)	Priority Claimed Under 35 U.S.C. §119

Prior United States Provisional Application(s)

We hereby claim priority benefits under Title 35, United States Code, §119(e)(1) of any U.S. provisional application listed below:

U.S. Provisional Application No.	Date of Filing (day month year)	Priority Claimed Under 35 U.S.C. §119(e)(1)
60/121,495	24 February 1999	Yes

Prior United States Application(s)

We hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Date of Filing (Day Month Year)	Status — Patented, Pending, Abandoned

Banner & Witcoff Ref. No. 03037.00003
Client Ref. No.

Power of Attorney

And we hereby appoint, both jointly and severally, as our attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith the practitioners at:

Customer Number 22907 (WDC)

Please address all correspondence and telephone communications to the address and telephone number for this Customer Number.

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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SEQUENCE LISTING

SEQ ID NO: 1 GLEISEEINEEDLKECFF

SEQ ID NO: 2 GLEISEEINEEDLKECFFDDME

SEQ ID NO: 3 VP-22 (Phelan et al., Nature Biotech 16:440-443, 1998, incorporated

by reference herein)

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SEQ ID NO: 5 RQIKIWFQNRRMKWKK (amide)